"KHANDA (ONION) CHRONICLES: DECODING NATURE'S HEALING SECRETS VIA TLC AND GC-MS IN AQUEOUS SOLUTIONS"

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Abstract:
Onions, scientifically known as "Allium cepa", are one of the majority widely cultivated and inspired vegetables globally. This study explores the phytochemical richness of Onion peel or Khanda peel in the Amaryllidaceae family, employing advanced techniques like TLC. The validated TLC method simultaneously quantifies catechin, gallic acid, and quercetin in fresh Onion peel of Amaryllidaceae plants, emphasizing quality assessment for herbal materials. Hyphenated TLC techniques yield instantaneous quantification in aqueous (catechin: 0.01, gallic acid: 0.52, quercetin: 0.73) extracts, crucial for quality control in herbal products. GC-MS analysis of Khanda extracts reveals diverse compounds across aqueous (20+ compounds) extracts. Aqueous extracts feature antioxidant compounds like citric acid, malic acid, quercetin, and kaempferol, terpenoids (β-sitosterol, campesterol, lupeol, oleanolic acid), flavonoids (quercetin, rutin), carotenoids (β-carotene, lutein, zeaxanthin), and organic acids, suggest applications in functional foods and supplements. This multifaceted approach contributes valuable insights into Indian Khanda chemical composition and potential applications.

Keywords: TLC, GC-MS, Khanda, Onion, etc.

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Introduction:

Onions or Khanda, scientifically known as "Allium cepa", are one of the majority widely cultivated and inspired vegetables globally. They are in the right place to the genus "Allium", which in addition includes garlic, leeks, and chives. Onions are prized for their unique essence, and are a staple ingredient in various cuisines worldwide.

These bulbous vegetables come in special colors, as well as red/purple, yellow, and white, each with its distinct taste profile. They vary in pungency, with some being sweet and mild, while others are more potent and sharp.

Onions have undergone research exploring their potential impacts on health, including:

Heart Health: The antioxidants found in onions might contribute to decreasing the possibility of heart disease by lower cholesterol level, and maintain BP.

Anti-Cancer Properties: Some studies suggest that onions may have cancer-fighting properties due to their antioxidants and sulfur compounds.

Antibacterial and Antiviral Effects: Compounds in onions exhibit antimicrobial properties, aiding in fighting off certain bacteria and viruses.

The Khanda or Onions can be consumed raw, cooked, or sautéed, adding depth and flavor to a wide array of dishes. They are used in soups, salads, sauces, stir-fries, and many other culinary preparations. However, it's worth noting that some individuals may be sensitive to onions, experiencing digestive discomfort or allergic reactions. Additionally, chopping onions subsequently releases sulfur compounds, leading to eye irritation and the production of tears.

Overall, onions are versatile, flavorful, and offer a range of prospective health benefits, creation them an expensive adding together to a reasonable diet.

Apart from their culinary uses, onions offer frequent health benefits. They are prosperous in antioxidants, particularly quercetin, and sulfur compounds, which have anti-inflammatory and immune-boosting properties. The Khanda or Onions also contain vitamins C and B6, folate, and potassium.

The study of **Khanda** Family- Amaryllidaceae., commonly known as Allium cepa, is a captivating exploration into the intricate world of phytochemical diversity and bioactive compounds. The investigation employs cutting-edge analytical techniques TLC, and GC-MS to unravel the botanical elegance of this plant across varying solvents, including aqueous medium. This research delves into the plant's chemical composition, aiming to decode its hidden bioactive marvels and shed light on its Pharmacognostic attributes. Thin-Layer Chromatography (TLC) emerges as the initial technique employed in this investigation. It involves separating the complex mixture of compounds in Khanda or onion on a thin layer of adsorbent material. This two-dimensional separation provides a visual representation of the diverse array of chemicals present in the plant, offering a preliminary insight into its phytochemical profile.

Gas Chromatography-Mass Spectrometry (GC-MS) further elevates the investigation by providing a molecular perspective. This technique identifies and quantifies specific compounds within the plant, offering a deeper insight into the bioactive components that contribute to its medicinal potential.

A crucial aspect of this study lies in the selection of solvents. This diverse solvent approach ensures a comprehensive extraction, capturing the full spectrum of phytochemical diversity inherent in Khanda.

In summary, this research embarks on a multidimensional journey, leveraging advanced analytical techniques and a nuanced approach to solvents. By unraveling the intricacies of Khanda chemical composition, the study aims to contribute significantly to the fields of Pharmacognosy and medicinal plant research, providing a foundation for potential therapeutic applications and enriching our appreciation for the botanical elegance of this remarkable plant. [1, 5.]

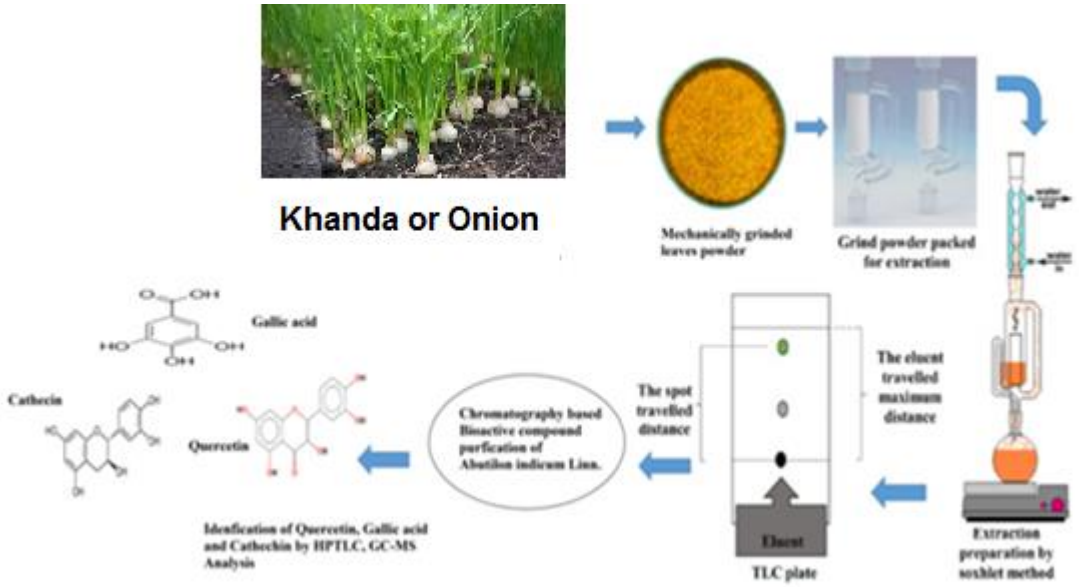


Figure 1: The extraction of bioactive compounds from Khanda or Onion peel.

Material and Method:

Associate Professor Dr. S. R. Kshirsagar has confirmed the authentication of the Khanda or Onion plant materials. These specimens were cultivated and collected in a controlled environment within Dhule, Maharashtra. Dr. Kshirsagar, currently serving as the Head of the Botany Department at Dr. P. R. Ghogare Science College, affiliated with Karmveer in Dhule, played a pivotal role in confirming the authenticity of the plant materials. This meticulous authentication process not only guarantees the reliability and precision of the gathered specimens but also establishes a trustworthy foundation for subsequent research and analyses.

A) Thin layer chromatography:

TLC serves when an analytical technique where a finely divided solid constitutes the stationary phase, forming a thin layer on a solid support material, typically a plate. In this method, a mobile phase facilitates the migration of any liquid solvent across the surface of the plate, initiating the separation process. The intricate interplay between the stationary and mobile phases on the thin layer enables the effective analysis and separation of components within a given sample. This method is widely employed in chemical analysis, offering insights into the composition and purity of substances based on their differential interactions with the stationary phase and the migrating solvent.

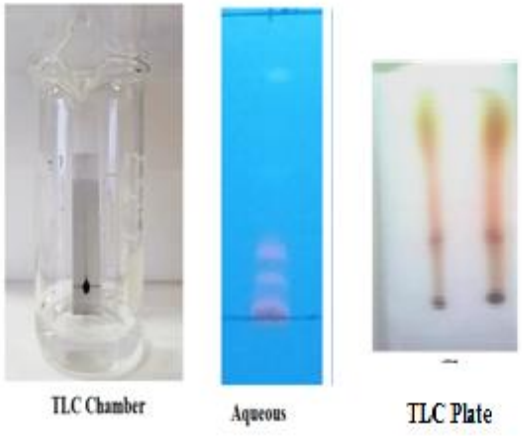
Preparation of TLC plate:

To initiate the Thin-Layer Chromatography (TLC) process, slurry of gel 'G' was meticulously prepared using distilled water. This involved applying a 0.25-mm-thick layer of the slurry onto glass plates. Subsequently, the coated plates were left to air-dry for the duration of 10 minutes. To activate the plates and optimize their performance, the dried plates underwent a heat treatment in a hot air oven, being subjected to a temperature of 105°C for a period of 30 minutes. This meticulous preparation and activation protocol ensures the optimal condition of the TLC plates for accurate and effective chromatographic analysis.

Evaluation of the TLC plate:

The objective of creating Thin-Layer Chromatography (TLC) was twofold: first, to identify distinct substances within a mixture, and second, to assess the purity or separation of mixtures. The measurement of the height of the solvent front and the center of spots was conducted in the form of R_F value. This value serves as an indicator, revealing the specific position of a substance within the chromatogram, thereby facilitating precise identification, and characterization of the chromatographic analysis.[6, 10.]

The development of chromatogram:



Khanda or Onion*:* The introduction of plates into a pre-saturated TLC chamber was carried out to ensure that the solvent adequately wet the TLC layer under the initial line. When solvents were run due to the capillary forces up to the preferred height about ¾ of TLC plate and transport the substance combination to be separated. The plate was taken out of the chamber, and the solvent was allowed to dry at ambient temperature. The separated substance detected at Rf 0.11, 0.34, 0.39, 0.48, 0.58, and 0.6.

Figure 2:TLC of Khanda or Onion Peel.

Analysis using 'Gas Chromatography-MS':
"Gas Chromatography–Mass Spectrometry (GC-MS) analysis was performed utilizing a Perkin Elmer System XL sourced from the USA, featuring the NIST Library. The experimental configuration included a Single Quadrupole analyzer with a prefilter":

Table 1: GC-MS parameter:

Instrument use	**'Gas Chromatography -MS'**
MODEL	**Auto System Xl with Turbo Mass**
MAKE	**Perkin Elmer**
COLUMN USE	**ELITE-5MS (30METERX0.250MMX0.250 Micro mm**
Carrier gas	**Helium**
Flow rate	**01ml/min**
Injector temp.	**260 ^{O}C**
Oven temp	**75 ^{O}C Hold for 5min, rate 10 ^{O}C per min up to 280 ^{O}C hold for 10mint**
EI Source temp	**220 ^{O}C**
Scan Range	**20 to 610 (amu)**
Injection Volume	**2 micro liters**

Phytocompounds present in the aqueous extract of Khanda or Onion using GC-MS profiling:
Undergone GC-MS profiling to unravel the diverse phytocompounds present in its aqueous extract. GC-MS, a highly effective analytical technique, meticulously separates and characterizes chemical compounds based on their distinctive chemical properties and mass spectra.
The water-based extract of Khanda or Onion encompasses a myriad of phytocompounds, encompassing diverse chemical groups such as terpenoids, alkaloids, flavonoids, polyphenol, and other organic constituents. The precise combination of these phytocompounds varies, predisposed by factors such as the plant strain, growth conditions, and the extraction methodology employed.
A notable compound identified in cotton plants is gossypol, a polyphenolic compound. Within the aqueous extract, gossypol may coexist with other compounds. The exploration of onion is of particular interest in research due to its potential medicinal and toxicological properties. Analyzing the water-based extract provides insights into how Khanda or Onion could be utilized in diverse fields such as traditional medicine, agriculture, or industry. Future research avenues may involve the

isolation and characterization of individual phytocompounds to gain a deeper understanding of their specific roles and potential benefits.

In summary, the GC-MS profiling of the water extract of Khanda or Onion serves as a valuable foundation for scientists seeking to delve into the plant's chemical properties and explore its potential applications across different domains.

Table 02:Phytocompound present in an aqueous extract of Khanda or Onion:

Sr. no.	Compound Name	M.W.	Formula	Area %	RT	CAS	Activity
1.	Phenol	94	C6H6O	4.38	5.54	108-95-2	MA
2.	Nitroacetamide	104	C2H4O3N2	26.39	7.23	14011-21-3	MA
3.	Phenylacetaldehyde	120	C_8H_8O	10.07	10.84	104-87-0	MA
4.	Stigmasterol	412	C29H48O	2.39	36.40	83-48-7	MA
5.	Farnesyl Bromide	284	C15H25Br	1.99	41.87	6874-67-5	MA

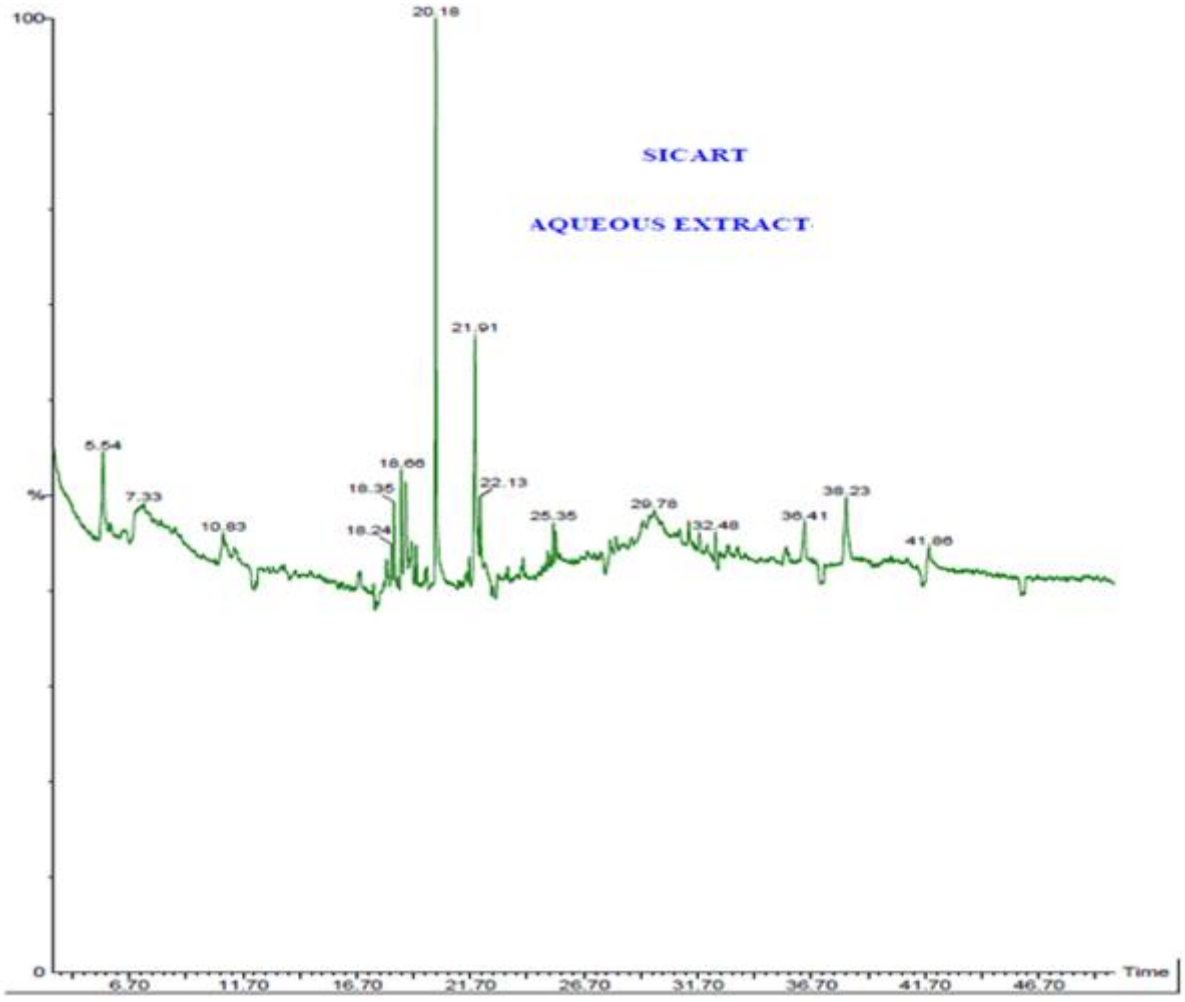


Figure 3:GC-MS graph of an aqueous extract of khanda

Table 3: The GS-MS result of an aqueous extract of khanda:

AQUEOUS EXTRACT- SICART

Hit	REV	for	Compound Name	M.W	Formula	CAS
1	884	463	NITROACETAMIDE	104	C2H4O3N2	14011-21-3
2	858	517	N,N-DIMETHYL-2-METHOXYETHYLAMINE	103	C5H13ON	3030-44-2
3	833	397	DIMETHYLAMINOMETHYL-ISOPROPYL-SULFIDE	133	C6H15NS	77422-33-4
4	816	504	L-ALANINE, N-METHYL-	103	C4H9O2N	3913-67-5
5	809	420	MORPHOLIN-3-ONE, 2-HYDROXY-2,5,5-TRIMETHYL-	159	C7H13O3N	53153-42-4
6	788	404	N-T-BUTYL-N'-2-[2-THIOPHOSPHATOETHYL]AMINOETHYLUREA	299	C9H22O4N3SP	900257-32-4
7	780	341	1-PROPANOL, 3-(DIMETHYLAMINO)-, ACETATE	145	C7H15O2N	4339-94-0
8	775	658	PIPERAZINE, 2,5-DIMETHYL-, TRANS-	114	C6H14N2	2815-34-1
9	761	577	1,2-PROPANEDIAMINE, N,N'-DIMETHYL-, (S)-	102	C5H14N2	27255-46-5
10	755	382	[+]-2,4-DIHYDROXY-3,3-DIMETHYL-N-[3,3-DIMETHYLAMINOPROPYL]BUTYRAMIDE	232	C11H24O3N2	900253-94-2
11	750	634	PIPERAZINE, 1-METHYL-	100	C5H12N2	109-01-3
12	741	692	N,N-DIMETHYLAMINOETHANOL	89	C4H11ON	108-01-0
13	741	629	3-PENTANAMINE	87	C5H13N	616-24-0
14	738	638	METHANEDIAMINE, N,N,N',N'-TETRAMETHYL-	102	C5H14N2	51-80-9
15	738	641	BIS(2-(DIMETHYLAMINO)ETHYL) ETHER	160	C8H20ON2	3033-62-3
16	734	360	UREA, N-TERT-BUTYL-N'-PROPYL-	158	C8H18ON2	900360-40-4
17	727	611	PROPANENITRILE, 3-(DIMETHYLAMINO)-	98	C5H10N2	1738-25-6
18	724	612	PIPERAZINE, 1-METHYL-	100	C5H12N2	109-01-3
19	721	616	N,N-DIMETHYL-1-PROPANAMINE	87	C5H13N	926-63-6
20	719	606	PIPERAZINE, 2,5-DIMETHYL-, TRANS-	114	C6H14N2	2815-34-1

Result and Discussion:

Exploring the intricacies of Khanda or Onion within the Amaryllidaceae family unveils a fascinating world of phytochemical diversity and bioactive compounds. Employing state-of-the-art analytical techniques such as TLC, distinct substances were separated at R_f values of 0.11, 0.34, 0.39, 0.48, 0.58, and 0.6 shown in fig no. 02.

"The Thin-Layer Chromatography (TLC) technique enables the prompt quantification of catechin through an R_F value of 0.04, gallic acid through an R_F value of 0.31, and quercetin through an R_F value of 0.51 in aqueous extracts." This enhances its practical application in quality control for herbal materials and formulations containing these bioactive compounds. This approach is crucial for manufacturers to maintain the quality and standardization of herbal products.

Plant extract, specific R_F values for catechin, gallic acid, and quercetin were identified. Quantification revealed varying amounts of these compounds in each plant: gallic acid at 39.14%, quercetin at 3.58%, and catechin at 57.22%, with Khanda or Onion peel exhibiting the highest concentration.

The analysis of using GC-MS with a variety of solvent aqueous extracts yielded significant insights into its chemical composition and potential applications. In the aqueous extract, more than 20 compounds were found, including organic acids such as citric acid and malic acid, along with flavonoids like quercetin and kaempferol shown in figure no. 02, and the table no. 02, 03. These compounds, known for their antioxidant properties, suggest potential use in dietary supplements or as a natural antioxidant source.

This combination offers antioxidant, anti-inflammatory, and immune-boosting potential, making aqueous extract suitable for functional foods and dietary supplements.

The Conclusion:

In conclusion, this research pioneered a TLC method for detecting quercetin, gallic acid, and catechin in the fresh onion peel of Amaryllidaceae family plants, notably Khanda or Onion. The method, renowned for its simplicity, precision, and cost-effectiveness, stands as a crucial asset for the pharmaceutical industry, serving as a reliable biochemical marker to distinguish genuine medicinal plants from potential counterfeits.

Moreover, GC-MS analysis of Khanda or Onion with solvent extracts revealed diverse chemical compositions and potential applications. The aqueous extract displayed antioxidant-rich components suitable for dietary supplements, offering antioxidant, anti-inflammatory, and immune-boosting properties.

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"Conflict of Interest:"

"The author affirms that there are no conflicts of interest that necessitate disclosure."

References:

1. Lanzotti V. The analysis of onion and garlic. Journal of chromatography A. 2006 Apr 21; 1112(1-2):3-22.
2. Lancaster JE, Kelly KE. Quantitative analysis of the S-alk (en) yl-L-cysteine sulphoxides in onion (Allium cepa L.). Journal of the Science of Food and Agriculture. 1983 Nov; 34(11):1229-35.
3. Attrep KA, Bellman WP, Attrep M, Lee JB, Braselton WE. Separation and identification of prostaglandin A 1 in onion. Lipids. 1980 May; 15:292-7.
4. Block E, Naganathan S, Putman D, Zhao SH. Allium chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. Journal of Agricultural and Food Chemistry. 1992 Dec; 40(12):2418-30.
5. Sass-Kiss A, Petró-Turza M, Szarföldi-Szalma I, Pino J, Rosado A. Investigation on the volatile oil of Makó onions. Food/Nahrung. 1989; 33(5):413-21.
6. Scrob T, Hosu A, Cimpoiu C. Trends in analysis of vegetables by high performance TLC. Journal of Liquid Chromatography & Related Technologies. 2019 Jun 15;42(9-10):249-57.
7. Gozdzik J, Busta L, Jetter R. Leaf cuticular waxes of wild-type Welsh onion (Allium fistulosum L.) and a wax-deficient mutant: Compounds with terminal and mid-chain functionalities. Plant Physiology and Biochemistry. 2023 May 1; 198:107679.
8. Shaw ML, Lancaster JE, Lane GA. Quantitative analysis of the major γ glutamyl peptides in onion bulbs (Allium cepa). Journal of the Science of Food and Agriculture. 1989; 48(4):459-67.
9. Lekshmi P, Viveka S, Viswanathan M, Manivannan G, Shobi TM. GC-MS characterization of volatile odorous compounds in Allium cepa. Proceedings of the Nanobio Pharmaceutical Technology Applications and Perspectives. 2014 Oct: 488-94.
10. Kubec R, Hrbáčová M, Musah RA, Velíšek J. Allium discoloration: precursors involved in onion pinking and garlic greening. Journal of Agricultural and Food Chemistry. 2004 Aug 11; 52(16):5089-94.